



DELAWARE HEALTH AND SOCIAL SERVICES
Division of Public Health
Laboratory

Delaware Public Health Laboratory Specimen Collection Procedures for Identification of Bioterrorism Organisms

DISEASE OR AGENT	SPECIMEN SELECTION FROM SAMPLES		TEST METHOD	TRANSPORT MEDIUM/ INSTRUCTIONS	PROCESSING TIME	COMMENTS
Anthrax <i>Bacillus anthracis</i>	Clinical	* Vesicular Stage : use sterile swabs to collect fluid from intact vesicles. The organism is best demonstrated in this stage. * Eschar stage : without removing eschar, insert swab beneath the edge of eschar, rotate and collect lesion material.	Culture*: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	
			PCR: available on grown organism only.	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		Lesion biopsy : collect biopsy in sterile tube with 10% formalin.	Histochemical stain: available through CDC for high suspect cases only	Double bag, sent at room temp	Will need to be sent to CDC	Contact DPHL to request CDC reference number and transportation options. May require Chain of Custody
		* Stool : collect 5-10 g in a clean, sterile, leak proof container.	Culture*: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	48-72 hrs	Minimal recovery
			PCR: available on grown organism only	Grown isolated on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		* Blood Culture : collect per institution's procedure for routine blood culture. Blood culture is incubated by facility drawing the culture and screened for growth of the suspect organism.	Culture*: not available	Grown isolate on agar plate sent at room temp	24-72 hrs	Blood culture is positive in late stages of disease Blood culture can not be performed at DPHL
			PCR available on grown organism only	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		Blood : purple top tube (EDTA), serum, plasma	Culture*: not available PCR: available on original specimen	Refrigerate at 2-8C	8 -24 hrs	May require Chain of Custody PCR requires culture confirmation
	Environmental	Powder, letter, etc. : collect at least two non-cotton swabs, place in separate sterile containers.	Culture: available	Dacron or polyester swabs or a culturette	24-72 hrs	Chain of Custody required
			PCR: available	Dacron or polyester swabs no cuturettes	4-8 hrs	Chain of Custody required PCR requires culture confirmation
	Food	Notification of DPHL by DPH Epidemiology Section is necessary as soon as possible due to the large amount of media necessary to process food.	Culture and PCR: available Send all suspect food double bagged in a sterile, leak proof container. Minimum needed to test: 25 grams	Refrigerated samples & those collected at room temperature should be transported at 4C. Transport frozen samples on dry ice.	2-5 days	Chain of Custody required PCR requires culture confirmation Submitter must contact DPH Epidemiology Section concerning any suspected food related infections.

*Cultures from clinical specimens should be performed by the sentinel laboratory



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	Specimen Type	Clinical Syndrome A= Autopsy specimen may be used				Mouse Bioassay is performed if less than 10cc of extracted supernatant available	Mouse Bioassay* is only confirmatory test available	Specimen(s) of choice for confirmation of botulism: Serum, wound/ tissue, stool and incriminated food
		Foodborne	Infant	Wound	Intentional Release***			
Botulism Toxins are produced by certain strains of <i>Clostridium botulinum</i>	Enema Fluid	X	X	X	X	20 cc Refrigerate at 4°C	Anaerobic Culture* Mouse Bioassay	Anaerobic Culture: organism is isolated and identified as Clostridium botulinum. Extraction of toxin from organism may be tested by DIG-ELISA and Mouse Bioassay. Mouse Bioassay: confirmatory test.
	Gastric Fluid	X, A				20 cc Refrigerate at 4°C	Anaerobic Culture* DIG-ELISA** Mouse Bioassay	Purge with a minimal amount of sterile nonbacteriostatic water to minimize dilution of toxin Collect up to 20 cc
	Intestinal Fluid	A	A			20 cc Refrigerate at 4°C	Anaerobic Culture* Mouse Bioassay	Autopsy: intestinal contents from various areas of the small and large intestines should be provided.
	Nasal Swab				X	Anaerobic Swab Room Temp	Anaerobic Culture* Mouse Bioassay	For aerosolized botulinum toxin exposure, obtain nasal culture for C. botulinum and serum for mouse toxicity testing.
	Serum	X, A		X	X	10-12 cc Refrigerate at 4°C	Mouse Bioassay	Serum should be obtained as soon as possible after the onset of symptoms and before antitoxin is given. A minimum of 10 cc of serum (20cc of whole blood) is required for mouse toxicity testing. In infants, serum is general not useful, since the toxin is quickly absorbed before the serum can be obtained.
	Stool	X	X	X	X	10-50 g Refrigerate at 4°C	Anaerobic Culture* DIG-ELISA** Mouse Bioassay	Botulism has been confirmed in infants with only “pea sized” stools. Please note: anticholinesterase given orally, in patients with myasthenia gravis, has been shown to interfere with toxin testing.
	Vomitus	X				20 cc Refrigerate at 4°C	Anaerobic Culture* Mouse Bioassay	Collect up to 20 cc
	Wound/ Tissue			X		Anaerobic Swab or Transport System Room Temp	Anaerobic Culture* Mouse Bioassay	Exudates, tissue, or swabs must be collected and transported in an anaerobic transport system. Samples from an enema or feces should also be submitted since the wound may not be the source of botulinum toxin.
	Environmental Sample**		X		X	3-6 Swabs Room Temp	Anaerobic Culture* DIG-ELISA** Mouse Bioassay	Environmental Swabs Chain of Custody required
	Food**	X	X		X	Culture: 25-50 g Refrigerate at 4°C DIG-ELISA: >5mL liquid or 25 grams of solid Refrigerate at 4°C	Anaerobic Culture* Mouse Bioassay DIG-ELISA**	Chain of Custody required Food that supports C. botulinum growth will have a pH of 3.5-7.0. The most common pH is 5.5-6.5. Submit food in original container, placing individually in leak proof sealed transport devices. Submitter must contact Epidemiology Section of DPH concerning any suspected food related infections.

*Anaerobic culture for suspected *C. botulinum* specimens is performed by LRN reference laboratory

**DPHL only performs DIG-ELISA testing on Environmental and Food samples for botulism. It will detect types A, B, E, & F. All other specimens will be sent to a reference laboratory.

**DIG-ELISA positive result is considered presumptive, the specimen must be sent to CDC for mouse bioassay for confirmation.

***Clinical specimens from an intentional release may require a Chain of Custody



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Brucellosis <i>Brucella melitensis</i> <i>Brucella abortus</i> <i>Brucella suis</i> <i>Brucella canis</i>	Clinical Acute, subacute, or chronic	*Bone marrow, spleen, or liver: collect per institution's surgical/pathology procedure.	Culture*: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	5-10 days	
			PCR: available on grown organism only.	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		Blood Culture: collect per institution's procedure for routine blood culture. Blood culture is incubated by facility drawing the culture and screened for growth of the organism.	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	5-10 days	Blood culture can not be performed at DPHL
			PCR: available on grown organism only.	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		Blood: whole blood, at least 200 ul	Culture*: not available			
			PCR available on grown organism only	Refrigerate at 2-8C Shipping requires freezing at -20°C	4-8 hrs	PCR requires culture confirmation May require Chain of Custody
	Environmental	Serum: collect 10-12 cc acute phase specimen as soon as possible after disease onset. Collect a convalescent specimen obtained 21 days later.	Serologic diagnosis: 1. Single titer greater than 1:160 2. 4-fold rise in titer 3. IgM positive	Refrigerate at 2-8C Shipping requires freezing at -20°C	Will need to be sent to CDC	Contact DPHL to request CDC reference number and transportation options May require Chain of Custody
		Powder, letter, etc.: collect at least two non-cotton swabs, place in separate sterile containers.	Culture: available PCR: available on original specimen and grown organisms	Use Dacron or polyester swabs or a culturette Use Dacron or polyester swabs, no culturettes	5-10 days 4-8 hrs for organism 8-24 hrs for environmental	Chain of Custody required Chain of Custody required PCR requires culture confirmation
	Food	Notification of DPHL by DPH Epidemiology Section is necessary as soon as possible due to the large amount of media necessary to process food.	Culture and PCR: available Send all suspect food double bagged in a sterile, leak proof container. Minimum needed to test: 25 grams	Refrigerated samples & those collected at room temperature should be transported at 4C. Frozen samples should be transported on dry ice.	5-10 days	Chain of Custody required PCR requires culture confirmation Submitter must contact DPH Epidemiology Section concerning any suspected food related infections.

*Cultures from clinical specimens should be performed by the sentinel laboratory



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Glanders <i>Burkholderia mallei</i> Melioidosis <i>Burkholderia pseudomallei</i>	Clinical	* Abscesses : collect using a culturette	Culture**: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	4-7 days	
			PCR*: available on grown organism only	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		* Urine, tissue aspirates : collect into a sterile, leak proof container.	Culture**: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	4-7 days	
			PCR*: available on grown organism only	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		* Blood Culture : collect per institution's procedure for routine blood culture. Blood culture is incubated by facility drawing the culture and screened for growth of the organism.	Culture**: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	4-7 days	Blood culture is rarely positive Blood culture can not be performed at DPHL
			PCR*: available on grown organism only	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		Blood : serum (at least 200 ul)	Culture: not available			
			PCR*: available on grown organism only	Refrigerate at 2-8°C Transport on dry ice	8-24 hrs	May require Chain of Custody PCR requires culture confirmation
	Environmental	Powder, letter, etc.: collect at least two non-cotton swabs, place in separate sterile containers.	Culture: available	Use Dacron or polyester swabs	8-24 hours	Chain of Custody required
			PCR*: available on original specimen and grown organisms	Use Dacron or polyester swabs	4-8 hrs for organism 8-24 hrs for environmental	Chain of Custody required PCR requires culture confirmation
	Food	No method available at DPHL or CDC				

*PCR detects both *Burkholderia mallei* and *Burkholderia pseudomallei*. Culture is necessary to differentiate between species.

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Q fever <i>Coxiella burnetti</i>	Clinical	2 Bloods in serum separator tube: 1 st tube: collect first tube as soon as possible within four days of illness 2 nd tube: collect about two to three weeks after onset of disease	Indirect Immunofluorescent antibody (IFA) test for detection of specific antibodies in serum	Refrigerate at 2-8°C	12-24 hours after initial set up	May require Chain of Custody
			Standard PCR: not available			
		EDTA blood collected in acute phase before antibiotic therapy	IFA: not available			
			Standard PCR: available Real-Time PCR: available	Refrigerate at 2-8°C	24-48 hours	May require Chain of Custody
		Affected tissues to include autopsy specimens or tissue biopsies, blood clots, and cell cultures (or cell culture supernatants), collect in sterile leak proof screw-capped containers	IFA: not available			
			Standard PCR: available	Refrigerate at 2-8° If lengthy delay before reaching lab, freeze at -70°C	24-48 hours	May require Chain of Custody
	Environmental	Powder, letter, etc.: collect at least two non-cotton swabs, place in separate sterile containers.	IFA: not available			
			Real-Time PCR: available on original specimen	Do not use any transport media Refrigerate at 2-8°C	4-8 hrs for organism 8-24 hrs for environmental	Chain of Custody required PCR requires culture confirmation
	Food	No method available at DPHL or CDC				



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DISEASE AND AGENT	SPECIMEN SELECTION FROM SAMPLES		TEST METHOD	TRANSPORT MEDIUM/ INSTRUCTIONS	PROCESSING TIME	COMMENTS
Ricin toxin Ricinine biomarker determination	Clinical	Urine: Collect minimum 25-50mL in plastic urine specimen cup with o-ring	LC/MS/MS: available*	Freeze at -20 to -80°C following IATA PI 650 & CDC Chemical Exposure Guidelines	12-36 hrs	*Method available through LRN network – anticipated at DPHL
Ricin toxin Ricin toxin is detected using the TRF method	Environmental	*Swabs (cotton, polyester, Dacron, rayon, or foam) *Wipes (non-cotton gauze, polyester blend, e.g., "Handi-Wipes") *Plant material in an envelope, paper, powder, water, soil (2.0±0.1g)	Time-resolved fluorescence assay (TRF): available	Transport at room temperature	8-24 hrs	*Do not collect samples in containers made of glass. If possible avoid collection from any glass surface. Chain of Custody required
	Food	*Food, drink: collect 2.0±0.1g, place in sterile, leak proof, plastic container NO GLASS CONTAINERS	Time-resolved fluorescence assay (TRF): available	Refrigerate at 2-8°C	8-24 hrs	*Do not collect samples in containers made of glass. If possible avoid collection from any glass surface. Chain of Custody required
Ricin communis DNA from the Ricin communis plant is detected using PCR method	Clinical	No method available at DPHL or CDC				
	Environmental	*Swabs (cotton, polyester, Dacron, rayon, or foam) *Wipes (non-cotton gauze, polyester blend, e.g., "Handi-Wipes") *Plant material in an envelope, paper, powder, water, soil (2.0±0.1g)	Real-Time PCR: available	Transport at room temperature	8-24 hrs	*Do not collect samples in containers made of glass. If possible avoid collection from any glass surface. Chain of Custody required
	Food	*Food, drink: collect 2.0±0.1g, place in sterile, leak proof, plastic container NO GLASS CONTAINERS	Real-Time PCR: available	Refrigerate at 2-8°C	8-24 hrs	*Do not collect samples in containers made of glass. If possible avoid collection from any glass surface. Chain of Custody required



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DISEASE AND AGENT	SPECIMEN SELECTION FROM SAMPLES		TEST METHOD	TRANSPORT MEDIUM/ INSTRUCTIONS	PROCESSING TIME	COMMENTS
SEB Staphylococcal Enterotoxin B	Clinical	No method available at DPHL or CDC				
	Environmental	*Swabs (cotton, polyester, Dacron, rayon, or foam). *Wipes (non-cotton gauze, polyester blend, e.g., "Handi-Wipes"). *Plant material in an envelope, paper, powder, water, soil (2.0±0.1g) in sterile (if possible), leak proof container. NO GLASS CONTAINERS	Time-resolved fluorescence assay (TRF): available	Place into a leak proof, sealed, non-glass transport device.	8-24 hrs	*Do not collect samples in containers made of glass. If possible avoid collection from any glass surface Chain of Custody required
	Food	*Food, drink: collect 2.0±0.1g, place in sterile (if possible), leak proof, plastic container. NO GLASS CONTAINERS	Time-resolved fluorescence assay (TRF): available	Place into a leak proof, sealed, non-glass transport device.	8-24 hrs	*Do not collect samples in containers made of glass. If possible avoid collection from any glass surface Chain of Custody required



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Tularemia <i>Francisella tularensis</i>	Clinical	*Bronchial/tracheal wash: collect per institution's procedure in an area dedicated to collection of respiratory specimens under isolation/containment circumstances, i.e., isolation chamber/"bubble"	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	
			PCR: available on grown organism only	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		*Sputum/throat: collect routine throat culture using a swab or collect expectorated sputum into a sterile, leak proof container.	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	Minimal recovery -- not recommended
			PCR: available on grown organism	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		*Blood Culture: collect per institution's procedure for routine blood culture. Blood culture is incubated by facility drawing the culture and screened for growth of the organism.	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	Blood culture is rarely positive Blood culture can not be performed at DPHL
			PCR: available on grown organism	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		*Ulcer/wounds/tissue: collect 2 swabs using a culturette, collect tissue in sterile container.	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	
			PCR: available on grown organism	Grown isolate on agar plate sent at room temp	4-8 hrs	May require Chain of Custody PCR requires culture confirmation
	Environmental	Blood (EDTA): one purple top tube	Culture: not available			
			PCR: available on original specimen	Refrigerate at 2-8°C	8 -24hrs	May require Chain of Custody PCR requires culture confirmation
		Powder, letter, etc.: collect at least two non-cotton swabs, place in separate sterile containers.	Culture: available	Use Dacron or polyester swabs or a culturette	2-7 days	Chain of Custody required
			PCR: available on original specimen and grown organisms	Use Dacron or polyester swabs, no culturettes	4-8 hrs for organism 8-24 hrs for environmental	Chain of Custody required on environmental samples PCR requires culture confirmation
	Food	Notification of DPHL by DPH Epidemiology Section is necessary as soon as possible due to the large amount of media necessary to process food.	Culture and PCR: available Send all suspect food double bagged in a sterile, leak proof container. Minimum needed to test: 25 grams	Refrigerated samples & those collected at room temperature should be transported at 4C. Transport frozen samples on dry ice.	2-7 days	Chain of Custody required PCR requires culture confirmation Submitter must contact DPH Epidemiology Section concerning any suspected food related infections.

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DISEASE AND AGENT	SPECIMEN SELECTION FROM SAMPLES		TEST METHOD	TRANSPORT MEDIUM/ INSTRUCTIONS	PROCESSING TIME	COMMENTS
Vaccinia virus “pox” type virus related to smallpox and is used to induce immunity against smallpox	Clinical	Dried vesicular fluid on a slide	Virology tests: none available			
			PCR: available	Slides must be packed in a plastic slide container Refrigerate at 2-8°C	12 hours from receipt of sample	NOTES: These results are preliminary. Positive PCR results must be confirmed by CDC. Clinical specimens may require Chain of Custody.
		Vesicular tissue (skin from roof of vesicle).	Virology tests: none available			
			PCR: available	Place in a sterile, leak proof, unbreakable container Refrigerate at 2-8°C	12 hours from receipt of sample	NOTES: These results are preliminary. Positive PCR results must be confirmed by CDC. Clinical specimens may require Chain of Custody.
		Cultured cellular material from tissue culture demonstrating cytopathic effect (PERFORMED ONLY WHEN VACCINIA IS SUSPECTED, NOT <u>SMALLPOX</u>)	Virology tests: none available			
			PCR: available	Place in a sterile, leak proof, unbreakable container Refrigerate at 2-8°C	12 hours from receipt of sample	NOTES: These results are preliminary. Positive PCR results must be confirmed by CDC. Clinical specimens may require Chain of Custody.
	Environmental	Swabs	Virology tests: none available			
			PCR: available	Do not use viral transport media Refrigerate at 2-8°C	8-24 hrs	NOTES: These results are preliminary. Positive PCR results must be confirmed by CDC. Chain of Custody required
	Food	No method available at DPHL or CDC				



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DISEASE AND AGENT	SPECIMEN SELECTION FROM SAMPLES		TEST METHOD	TRANSPORT MEDIUM/ INSTRUCTIONS	PROCESSING TIME	COMMENTS
Chicken pox virus Varicella zoster virus (VZV) used as a rule-out test for low-moderate risk patients for possible smallpox	Clinical	Vesicle fluid or lesions: collect vesicular fluid on a polyester swab, scabs from a crusted lesion, skin from the top of a vesicle or pustule	Direct DFA: available	Refrigerate at 2-8°C	12-24 hrs	
			Viral culture: appropriate cell line will be inoculated at DPHL	Refrigerate at 2-8°C	4-10 days	
			PCR: available	Do not use viral transport media Transport at room temperature	8-12 hrs	PCR requires viral culture confirmation.
		Blood: 10 cc of blood collected in a serum separator tube	IFA: available	Refrigerate at 2-8°C	12-24 hrs	
			Viral culture: not available			
			PCR: not available			
	Environmental	No method available at DPHL or CDC				
	Food	No method available at DPHL or CDC				



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DISEASE AND AGENT	SPECIMEN SELECTION FROM SAMPLES ***Utilize CDC Guide D Precautions for collection of specimens from High-risk patients***		TEST METHOD	TRANSPORT MEDIUM/ INSTRUCTIONS	PROCESSING TIME	COMMENTS
Smallpox virus <i>Variola major</i> *NOTE: High risk patient testing (see other chart for low to moderate risk patient testing)	Clinical For patients in the high risk categories of the rash illness protocol: 1. A suspected case of small pox should be reported immediately to DPH for review. 2. And if, after review, a true case of smallpox is still suspected, DPH will contact CDC's Poxvirus Section for approval to send specimens. 3. DPHL will contact CDC for review of the packaging/shipping requirements and to seek coordination of transport/shipment of the specimens to CDC.	Fresh lesions: if possible, collect several Dacron or polyester swabs, place each swab in separate viral transport media	CDC will determine tests to be performed	Refrigerate at 2-8°C	CDC will notify DPHL of results. DPHL will then notify submitter as soon as possible.	*Specimens from patients in the high-risk category for possible smallpox will not be tested at DPHL. CDC requires BioSafety Level 4 facilities to be used for smallpox testing and specimens will therefore be referred to CDC for testing. Clinical Specimens may require Chain of Custody.
		Autopsy specimens: Including portions of skin containing lesions, liver, kidney, etc., for virus isolation should be frozen	CDC will determine tests to be performed	Ship frozen on dry ice		
		Biopsy: Place dried vesicular fluid on a slide ("touch prep")	CDC will determine tests to be performed	Package in slide box, do not freeze.		
		Vesicles or pustules: use scalpel to open, remove top of the vesicle or pustule and place the skin of the vesicle top into a 1.5-2.0 ml screw capped plastic vial.	CDC will determine tests to be performed	Refrigerate at 2-8°C		
		Scab lesions: place two scabs in each of two 1.5-2.0 ml screw capped plastic vial.	CDC will determine tests to be performed	Refrigerate at 2-8°C		
		Blood: 10 cc of blood collected in a serum separator tube	CDC will determine tests to be performed	Refrigerate at 2-8°C		
	Environmental	No method available at DPHL or CDC				
	Food	No method available at DPHL or CDC				

***CDC Smallpox Draft Guide C, Part 1 Infection Control Measures for Healthcare and Community Settings, Guide F – Environmental Control of Smallpox Virus, <http://www.bt.cdc.gov/agent/smallpox/infection-control/>



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Smallpox virus <i>Variola major</i> NOTE: Low risk patient testing only (see other chart for high risk patient testing)	Clinical For patients in the low and moderate risk categories according to the evaluation criteria in the CDC rash illness protocol: DPHL can perform rule-out tests for Smallpox, which includes vaccinia, VZV (Varicella-zoster virus), and herpes simplex virus. DO NOT ORDER THESE TESTS IF PATIENT FALLS INTO HIGH RISK CATEGORY. REFER TO THE SMALLPOX HIGH RISK CHART!!!!	Fresh lesions: if possible, collect several Dacron or polyester swabs, place each swab in separate viral transport media	Cell culture	Refrigerate at 2-8°C	4-10 days	For patients in low and moderate risk categories according to the evaluation criteria in the CDC rash illness protocol DPHL can perform rule-out tests for Smallpox, which includes vaccinia, VZV (Varicella-zoster virus), and herpes simplex virus. Clinical Specimens may require Chain of Custody.
		Autopsy specimens: Including portions of skin containing lesions, liver, kidney, etc., for virus isolation should be frozen	Cell culture	Ship frozen on dry ice	4-10 days	
		Biopsy: Place dried vesicular fluid on a slide ("touch prep")	PCR	Package in slide box, do not freeze.	12 hours from receipt of sample	
		Vesicles or pustules: use scalpel to open, remove top of the vesicle or pustule and place the skin of the vesicle top into a 1.5-2.0 ml screw capped plastic vial.	Cell culture	Refrigerate at 2-8°C	4-10 days	See above. PCR performed at CDC.
		Scab lesions: place two scabs in each of two 1.5-2.0 ml screw capped plastic vial.	Cell culture	Refrigerate at 2-8°C	4-10 days	See above.
		Blood: 10 cc of blood collected in a serum separator tube	IFA test for detection of specific antibodies in serum	Refrigerate at 2-8°C	2-24 hours after initial set up	See above.
	Environmental	No method available at DPHL or CDC				
	Food	No method available at DPHL or CDC				

***CDC Smallpox Draft Guide C, Part 1 Infection Control Measures for Healthcare and Community Settings, Guide F – Environmental Control of Smallpox Virus, <http://www.bt.cdc.gov/agent/smallpox/infection-control/>



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VHF Viral hemorrhagic fevers	Clinical	Blood: 10 -12 cc of serum in a red/black top tube	Send to CDC CDC will determine which tests to be performed Proper PPE including gloves should be worn while collecting specimen	Decontaminate outside of tube, place in a double leak proof container	CDC will notify DPHL of results. DPHL will then notify submitter as soon as possible.	CDC requires BioSafety Level 4 facilities to be used for testing for these viruses. DPHL does not have a BioSafety Level 4 Lab to test for these viruses. All submitted specimens will therefore be referred to CDC for testing. Clinical specimens may require Chain of Custody.
	Environmental	No method available at DPHL or CDC				
	Food	No method available at DPHL or CDC				



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Plague <i>Yersinia pestis</i>	Clinical	**Bronchial/tracheal wash**: collect per institution's in an area dedicated to collection of respiratory specimens under isolation/containment circumstances, i.e., isolation chamber/"bubble"	Culture*: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	
			PCR: available on grown organism only	Refrigerate at 2-8°C	8-24 hrs	May require Chain of Custody PCR requires culture confirmation
		Sputum/throat: collect routine throat culture using a swab or collect expectorated sputum into a sterile, leak proof container.	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	Minimal recovery -- not recommended
			PCR: available on grown organism	Refrigerate at 2-8°C	8-24 hrs	May require Chain of Custody PCR requires culture confirmation
		Blood Culture: collect per institution's procedure for routine blood culture. Blood culture is incubated by facility drawing the culture and screened for growth of the organism.	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	Blood culture is rarely positive Blood culture can not be performed at DPHL
			PCR: available on grown organism	Grown isolate on agar plate sent at room temp	4-8 hrs	PCR requires culture confirmation
		Nasopharyngeal swabs: for PCR the swabs must be collected in a sterile tube without transport media	Culture: rule-out and culture confirmation available on grown organism.	Grown isolate on agar plate sent at room temp	24-72 hrs	
			PCR: available on grown organism	Refrigerate at 2-8°C	8-24 hrs	May require Chain of Custody PCR requires culture confirmation
	Environmental	Powder, letter, etc.: collect at least two non-cotton swabs, place in separate sterile containers.	Culture: available	Use Dacron or polyester swabs or a culturette	8-24 hours	Chain of Custody required
			PCR: available on original specimen and grown organisms	Use Dacron or polyester swabs, no culturettes	4-8 hrs for organism 8-24 hrs for environmental	Chain of Custody required PCR requires culture confirmation
	Food	Notification of DPHL by DPH Epidemiology Section is necessary as soon as possible due to the large amount of media necessary to process food.	Culture and PCR: available Send all suspect food double bagged in a sterile, leak proof container. Minimum needed to test: 25 grams	Refrigerated samples & those collected at room temperature should be transported at 4C. Frozen samples should be transported on dry ice.	2-7 days	Chain of Custody required PCR requires culture confirmation Submitter must contact DPH Epidemiology Section concerning any suspected food related infections.

*Cultures from clinical specimens should be performed by the sentinel laboratory if possible